Report

Current Biology

Functional Divergence of Type 2 Deiodinase Paralogs in the Atlantic Salmon

Highlights

- The *Salmo salar* genome harbors six deiodinase paralogs
- Two type 2 deiodinase (*dio2*) paralogs diverged within the salmonid lineage
- *Dio2a* expression responds to seawater exposure and *dio2b* to photoperiod
- *Dio2a* in the gill is associated with the metabolic response to osmotic stress

Authors

Marlene Lorgen, Elisa Casadei, ..., David G. Hazlerigg, Samuel A.M. Martin

Correspondence

david.hazlerigg@uit.no

In Brief

Type 2 deiodinases activate thyroid hormone, controlling development and cell metabolism. Here, Lorgen et al. show that genome duplication has generated functionally divergent deiodinase paralogs in salmon, with differing sensitivity to day length and salinity. This refines timing of tissue-specific thyroid action prior to seaward migration.

Accession Numbers

KP851703
KP851704
KP851705
KP851706
KP851707
KP851708

Lorgen et al., 2015, Current Biology *25*, 936–941
March 30, 2015
http://dx.doi.org/10.1016/j.cub.2015.01.074

CellPress

CellPress

Current Biology
Report

Functional Divergence of Type 2 Deiodinase Paralogs in the Atlantic Salmon

Marlene Lorgen,[1] Elisa Casadei,[1] Elżbieta Król,[1] Alex Douglas,[1] Mike J. Birnie,[1] Lars O.E. Ebbesson,[2] Tom O. Nilsen,[2] William C. Jordan,[3,6] Even H. Jørgensen,[4] Hugues Dardente,[5] David G. Hazlerigg,[1,4,*] and Samuel A.M. Martin[1]
[1]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 2TZ, UK
[2]Uni Research Environment, Uni Research AS, Thormøhlensgt 49B, 5006 Bergen, Norway
[3]Zoological Society London, Institute of Zoology, London NW1 4RY, UK
[4]Department of Arctic and Marine Biology, Faculty of BioSciences, Fisheries and Economy, University of Tromsø, 9037 Tromsø, Norway
[5]INRA UMR85, CNRS UMR7247, Université de Tours, IFCE, 37380 Nouzilly, France
[6]Died May 25, 2011
*Correspondence: david.hazlerigg@uit.no
http://dx.doi.org/10.1016/j.cub.2015.01.074

SUMMARY

Thyroid hormone (TH) is an ancestral signal linked to seasonal life history transitions throughout vertebrates. TH action depends upon tissue-localized regulation of levels of active TH (triiodothyronine, T3), through spatiotemporal expression of thyroid hormone deiodinase (*dio*) genes. We investigated the *dio* gene family in juvenile Atlantic salmon (*Salmo salar*) parr, which prepare for seaward migration in the spring (smoltification) through TH-dependent changes in physiology. We identified two type 2 deiodinase paralogs, *dio2a* and *dio2b*, responsible for conversion of thyroxine (T4) to T3. During smoltification, *dio2b* was induced in the brain and gills in zones of cell proliferation following increasing day length. Contrastingly, *dio2a* expression was induced in the gills by transfer to salt water (SW), with the magnitude of the response proportional to the plasma chloride level. This response reflected a selective enrichment for osmotic response elements (OREs) in the *dio2a* promoter region. Transcriptomic profiling of gill tissue from fish transferred to SW plus or minus the deiodinase inhibitor, iopanoic acid, revealed SW-induced increases in cellular respiration as the principal consequence of gill dio2 activity. Divergent evolution of *dio2* paralogs supports organ-specific timing of the TH-dependent events governing the phenotypic plasticity required for migration to sea.

RESULTS AND DISCUSSION

Six Deiodinase Paralogs in the Atlantic Salmon

Thyroid hormone (TH) has long been implicated in seasonal and developmental physiology in salmonids [1, 2], but changes in circulating TH titers often correlate weakly with the timing of thyroid-dependent life history transitions [3, 4]. This has led to the suggestion that, as in birds and mammals, local tissue metabolism of TH by deiodinase (dio) enzymes may be a key aspect of salmonid endocrinology [5]. We surveyed the Atlantic salmon genome (NCBI: AGKD030000000) and found six distinct loci encoding *dio* paralogs. This complexity reflects a relatively recent (approximately 80–100 million years ago [6, 7]) whole-genome duplication (WGD) event during salmonid evolution.

Based on predicted amino acid homology, these paralogs represented one member of the vertebrate *dio1* family, two of *dio2* (designated *dio2a* and *dio2b*), and three of *dio3* (designated *dio3a1*, *dio3a2*, and *dio3b*) (Figure S1). Members of the *dio2* family encode selective, outer ring deiodinases responsible for conversion of thyroxine (T4) to active triiodothyronine (T3) within TH target tissues. The presence of only a single *dio2* gene in the Northern pike (*Esox lucius*), which represents the closely related sister group to salmonids, the Ecosiformes [8], indicates that salmon *dio2a* and *dio2b* diverged following WGD within the salmonid lineage. Members of the *dio3* family serve the converse function to *dio2*, encoding selective, inner ring deiodinases responsible for tissue conversion of T4 to inactive reverse T3 (rT3), or inactivating T3 by converting it further to diiodothyronine (T2). Although, similar to the *dio2* paralogs, salmon *dio3a1/2* diverged recently, divergence from *dio3b* appears to have occurred earlier, possibly reflecting earlier gene or WGD events in the teleost lineage. In contrast to either *dio2* or *dio3*, the *dio1* family encodes low selectivity enzymes with both outer and inner ring deiodinase activity [9]; this low selectivity may account for the lack of *dio1* paralog retention in the Atlantic salmon genome.

In mammals and birds, increased hypothalamic expression of *dio2* in the springtime drives a range of responses dependent on life history, including puberty or cyclical reactivation of the reproductive axis (or its inactivation in short-day breeders such as sheep), termination of the hibernation season, and increases in appetite (reviewed in [10–12]). We wondered whether a similar regulatory pathway might be involved in springtime migration in the juvenile Atlantic salmon. Here, the juvenile phase is characterized by slow growth and development as "parr" in nutrient-poor freshwater streams for the first 2–3 years of life, before a transition to a migratory "smolt," which heads downriver to sea. This so-called smoltification typically occurs in a

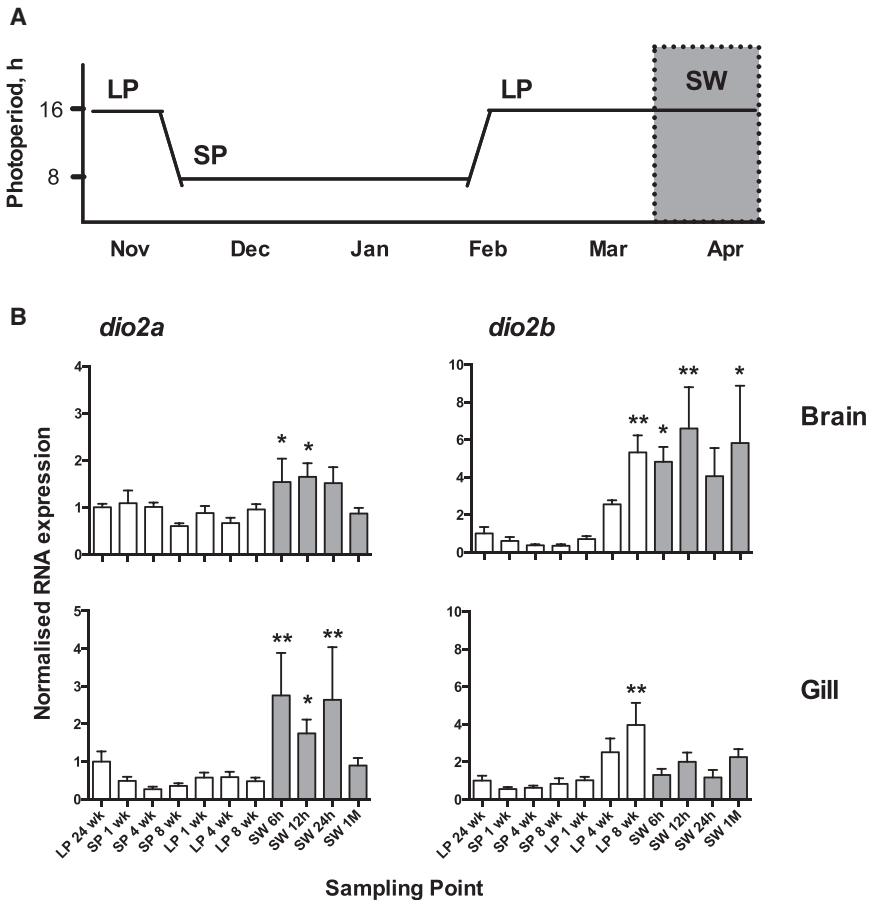

Figure 1. Differential Control of *dio2* Paralog Gene Expression in the Brain and Gill during Photoperiod-Induced Smoltification

(A) Experimental design.

(B) Brain and gill RNA expression levels at the sampling times indicated. *dio2* expression was normalized to housekeeping gene expression levels as described in Supplemental Experimental Procedures. Data are mean ± SEM of n = 5 individuals per sampling point, expressed relative to expression levels at the start of the experiment (LP 24 weeks). Asterisks (* and **) indicate significantly increased expression relative to values for SP 4 weeks ($p < 0.05$ and $p < 0.01$, respectively).

narrow time window in the spring. This is partly a response to increasing photoperiod and entails a range of preparative phenotypic changes including brain development and ion exchange capacity in the skin and gills [13].

To explore *dio2* expression during smoltification, we followed a standard protocol for inducing smoltification under laboratory conditions. This involved holding parr in freshwater (FW) at constant temperature (7°C), exposing them to short photoperiods (SP; 8 hr light/day) for 2 months, and then transferring them to long photoperiods (LP; 16 hr light/day) for 1 month before transferring them to artificial seawater (SW) (Figure 1A). Dramatically increased relative expression of the Na^+/K^+ ATPase α1b subunit [14] confirmed that this sequence of photoperiod exposure effectively mimicked the natural day length sequence, generating an SW-tolerant smolt phenotype (Figure S2). We focused on *dio2* expression in the brain and in the gills, since these organs are noted for sensitivity to photoperiod and salinity, respectively.

Photoperiodic Induction of *dio2b* Expression in Zones of Cell Proliferation in the Brain

In the brain, *dio2b* showed a significant response during photoperiod-induced smoltification ($p < 0.001$ by one-way ANOVA) (Figure 1B). Minimal levels of *dio2b* expression were observed after 4 weeks exposure to SP, rising some 6-fold by 8 weeks of exposure to LP. No further changes in brain *dio2b* expression were observed following subsequent transfer of LP-exposed fish to SW. Contrastingly, brain *dio2a* expression was not affected by the photoperiod manipulation phase of the smoltification protocol but showed a modest and transient increase upon exposure to SW ($p < 0.05$).

In situ hybridization analysis of parallel brain samples revealed a circumventricular distribution of expression of *dio2b* (Figure 2A), with high mRNA levels seen in the thalamus (T), hypothalamus, and the optic tectum (OT). Around both the third and lateral ventricles, expression varied strongly across the sampling schedule, being weakest in the tissue collected during exposure to SP and strongest after 8 weeks of LP exposure. Hence, changes in periventricular *dio2b* expression account for the observed effects on overall brain *dio2b* levels. We did not detect localized *dio2a* expression by in situ hybridization, probably reflecting low overall levels of expression of this gene in the brain. The sites of observed *dio2b* expression have previously been identified as cell-proliferative zones [15–17], as demonstrated by staining with proliferating cell nuclear antigen (PCNA) (Figures 2B–2D). During smoltification, TH-dependent neurogenesis appears important for the development of the adrenocorticotrophic axis and for the development of the olfactory system, both of which are critical for expression of the smolt phenotype [18, 19]. Our data strongly suggest that photoperiodic induction of *dio2b* provides a mechanism to promote coordinated changes in TH-dependent brain development during smoltification.

Differential Effects of Salinity on *dio2* RNA Expression in the Gill

Induction of *dio2b* by LP was also observed in the gill ($p < 0.001$ by one-way ANOVA), and similar to the brain, this peaked at 8 weeks. Contrastingly, expression of *dio2a* remained uniformly low in the gill tissue in fish maintained in FW (i.e., there was no evidence of day length-related changes in *dio2a* expression in either tissue) (Figure 1B) but increased dramatically following transfer to SW ($p < 0.01$). This response peaked by 6 hr of SW exposure, and by 1 month, levels were not significantly different to those prior to SW exposure.

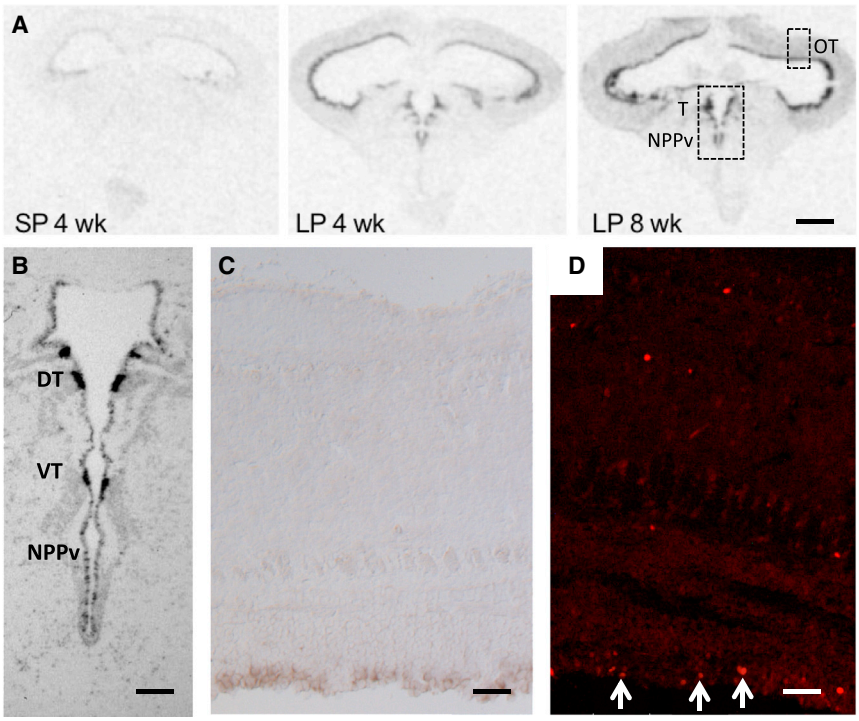

Figure 2. Photoperiodic Induction of Brain *dio2b* Expression Localizes to Circumventricular Cell-Proliferative Zones

(A) Representative images of radioactive in situ hybridization of *dio2b* expression in animals sampled under SP and after 4 or 8 weeks of LP exposure. Note intense expression surrounding the brain ventricles in LP-exposed fish. OT, optic tectum; T, thalamus; NPPv, ventral periventricular preoptic nucleus. The scale bar represents 1 mm.

(B) Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) in circumventricular cells in the thalamic region outlined in (A). DT, dorsal thalamus; VT, ventral thalamus.

(C and D) In situ hybridization of *dio2b* RNA and fluorescence immunohistochemical PCNA staining, respectively, in the region of the OT highlighted in (A); arrows indicate individual PCNA-labeled cell nuclei. The scale bar represents 50 μm.

To understand these differences in *dio2* regulation, we investigated the presence of transcriptional response elements in the proximal promoter regions of the *dio2a* and *dio2b* genes. This revealed two salient features. First, we found cyclic AMP (cAMP)-response elements (CREs; sequence 5′-TGACGTCA-3′) in the close vicinity of the predicted transcription start sites (TSSs; determined empirically by rapid amplification of 5′ cDNA ends) of both *dio2* genes, both of which were found to be functional in luciferase reporter experiments (Figures 3A and 3B). This site corresponds to the highly conserved proximal CRE, which is responsible for photoperiodic *dio2* induction in birds [20]. The fact that both *dio2a* and *dio2b* harbor a functional CRE within their proximal promoter, whereas only *dio2b* responds to long days, suggests that additional elements may modulate photoperiodic sensitivity.

Second, we found different numbers of canonical osmotic response elements (OREs; GGAAA[A/T][T/A/G] [21]) in the proximal promoter of the two *dio2* paralogs (Figure 3A). These elements have previously been linked to salinity adaptation in teleosts, mediating the effects of osmotically sensitive ORE binding proteins, and in the killifish (*Fundulus heteroclitus*), a single *dio2* gene has been identified, containing two OREs in the proximal 1.1 kb of promoter region [22]. In *S. salar dio2a*, we identified four canonical OREs, three lying within 0.5 kb upstream of the TSS and the fourth in the 5′UTR. This is more than ten times higher than the expected frequency of this response element, which is less than once every 2.5 kb. By contrast, no OREs were observed in the proximal 1-kb upstream region of the *S. salar dio2b* gene.

We used an in vivo approach to test the hypothesis that *dio2a*, but not *dio2b*, is directly sensitive to osmotic stress following transfer to SW. This involved challenging SP-acclimated fish to SW for 24 hr prior to assay of plasma chloride concentration and gill *dio2* RNA expression. Since the capacity to hypo-osmoregulate (i.e., to compensate for ionic influx and water loss) depends on the duration of SP acclimation (Figure S3), this produced a range of plasma Cl^- concentrations. We found a strong correlation between the magnitude of *dio2a* induction and plasma Cl^- concentration ($r^2 = 0.408$, $p < 0.0001$), whereas no relationship between Cl^- concentration and *dio2b* levels was observed (Figure 3C). Taken together, these observations support the hypothesis that gill *dio2a* induction is an osmotic stress response to SW exposure.

Functional Significance of Gill *dio2a* Induction

To gain insight into the functional significance of SW-induced *dio2a* induction, we performed a SW challenge experiment on LP-acclimated smolts, in which we inhibited gill dio2 activity (i.e., conversion of T4 to T3) by addition of iopanoic acid (IOP) to the SW. We also assessed the ability of co-treatment with T3 to override IOP effects. As before, SW exposure for 6 hr induced *dio2a* expression, while having no detectable influence on *dio2b* (Figure S4). Using a previously validated customized salmon microarray [23], we identified 1,939 genes whose expression was significantly ($p < 0.05$) increased or decreased by transfer from FW to SW for 6 hr. For a subset of 259 genes, this SW response was abolished if IOP was added to the SW, but maintained if T3 was also present during IOP treatment. This group of genes constitutes a candidate list, for which SW inducibility appears to be dependent on locally mediated changes in gill T3 availability. We performed pathway analysis on this group to identify the likely consequences of their altered expression following SW exposure. This revealed 25 pathways that were significantly overrepresented ($p < 0.05$), of which the most strongly enriched were mitochondrial dysfunction ($p < 0.001$) and creatine-phosphate biosynthesis ($p < 0.001$) (Table S1). Hence, the T3-dependent facet of the gill response to SW exposure is strongly associated with pathways to support increased energy expenditure through anaerobic and mitochondrial respiration.

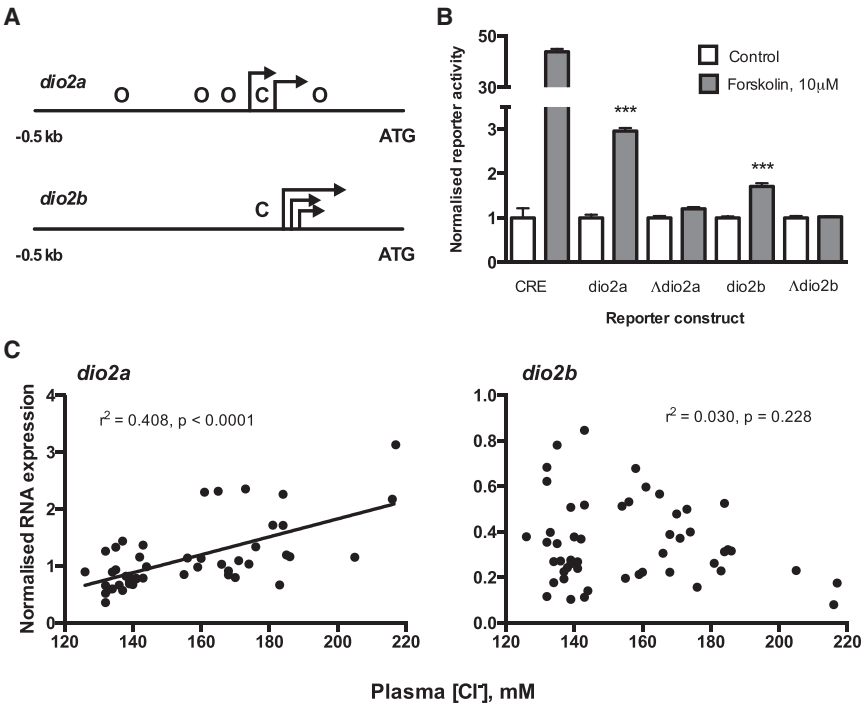

Figure 3. cAMP- and SW-Dependent Control of *dio2* Gene Expression

(A) Schematic diagram of proximal promoter regions of *dio2a* and *dio2b* genes. The figure shows location of canonical osmoregulatory elements (O) and cAMP response elements (C) in relation to transcription start sites as determined by 5′RACE PCR.

(B) The adenylate cyclase activator, forskolin (10 μM) induces expression of luciferase reporter constructs containing the proximal 1-kb promoter region of both the *dio2a* and *dio2b* genes. Forskolin induction is abrogated upon mutation of the CRE (Δdio2a and Δdio2b). Asterisks (***) indicate significantly increased expression compared to corresponding control values, $p < 0.001$.

(C) Positive correlation between plasma Cl^- concentration and *dio2a* expression following SW challenge of SP-acclimated juvenile Atlantic salmon (not observed for *dio2b*).

This outcome most likely reflects the high energy expenditure entailed in active transport of ions out of the gills, which is necessary for survival in SW. Specialized, mitochondrially enriched "SW chloride cells" serve this function, and these proliferate in the gill filaments during the preparatory, FW phase of smoltification [13] (Figure 4). These SW chloride cells must remain inactive until smolts reach the sea and apparently remain beneath the surface of the gill lamellae until entry to SW. According to this scheme, thyroid-dependent metabolic activation reflects mobilization of respiratory pathways to support ion transport in the initial phase of SW exposure, while the subsequent drop off in *dio2a* expression reflects completion of the activational phase in the transformation to an SW-tolerant gill phenotype.

Conclusions

Two basic conclusions emerge from this study. First, as in birds and mammals and echoing other recent reports in teleosts [24, 25], photoperiodic induction of *dio2b* gene expression is seen during smoltification, consistent with the view that this is an ancestral mechanism involved in the timing of seasonal life history transitions. This applies both to cyclical changes such as seasonal breeding as well as to seasonally gated unidirectional events such as puberty and metamorphosis, or in this case, smoltification. The distribution of *dio2b* expression seen in the smolt brain is far more extensive than that observed in adult mammals and birds, in which effects are confined to the pituitary and basal diencephalon. This probably reflects the widespread reorganization of brain function entailed in smoltification [18, 26], which involves profound, quasi-metamorphic [27–29] changes in behavior and physiology. At the same time, it forces us to reconsider the regulatory pathways leading to photoperiodic *dio2* induction. The current literature, including the recent work in teleosts, as well as that in birds and mammals, emphasizes the importance of thyrotropin (TSH) produced in the anterior pituitary [30, 31] or in the saccus vasculosus (SV) [24] as the upstream signal for *dio2* induction. The demonstration of changes in brain *dio2b* expression in sites remote from the pituitary or SV and the lack of evidence for extra-pituitary sites of TSH expression suggest that additional upstream pathways must be sought. One possibility that merits further attention, based on similarities in melatonin receptor distribution in the brain of another migratory salmonid (the rainbow trout, *Oncorhynchus mykiss*) [32] and *dio2b* gene expression in *S. salar* in the present study, is that melatonin mediates photoperiodic effects on brain *dio2b* expression during smoltification.

The second conclusion concerns functional divergence in the control by environmental cues through promoter rearrangement following gene duplication [33]. In mammals and birds, only a single *dio2* gene is present, and spatiotemporal control of TH action is achieved by directing cAMP-mediated signals to induce *dio2* expression through localized ligand-receptor interactions. Prominent examples include paracrine TSH signaling between the pituitary and the basal hypothalamus to drive photoperiodic induction of *dio2* in mammals and birds [34] and noradrenergic signaling in brown fat, which increases *dio2* expression as part of the thermogenic response [35]. In teleosts, *dio2* may sense osmotic changes through OREs [22], expanding the potential repertoire of routes to environmental regulation of *dio2*. Our finding that *S. salar* retains two *dio2* paralogs, *dio2a* and *dio2b*, specialized for salt- and light-dependent regulation, respectively, suggests that teleosts have taken a distinctive route to refinement of local TH action. In *S. salar*, the evolutionary narrative for *dio2* functional divergence is clear: smoltification first requires preparative developmental changes, cued by light, and final completion depends on SW responses upon entering the sea. The use of two routes to *dio2* induction gives precise timing in both the preparative and activational phases of smoltification. It has been suggested that the success of salmonids in using anadromy to exploit FW and SW environments [36, 37] derives from WGD within this lineage and the resultant potential for

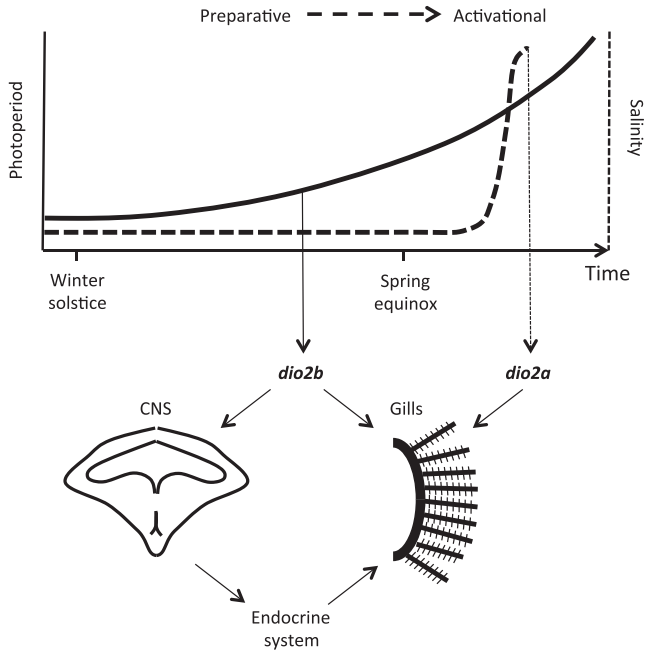

Figure 4. Interaction between Preparative and Activational Effects of TH Mediated by Differential Regulation of *dio2* Paralogs

The preparative phase of smoltification occurs in FW with full activation of the smolt phenotype being delayed until entry to SW. The model proposes that the timing of TH-dependent preparative effects, both in the CNS and in the peripheral organism, is determined by light-dependent regulation of *dio2b* expression. Indirect effects of brain *dio2b* changes occur via influences of TH on the brain-pituitary axis. TH-dependent activational effects, among which upregulation of gill metabolism to support hypo-osmoregulation is a major aspect, occur following initial transfer to SW and are mediated by osmotically sensitive *dio2a* expression.

functional divergence of gene duplicates. Potentially, the *dio2* paralog divergence reported here is one physiologically important instance of this phenomenon. Further comparative work is needed to test this interpretation.

ACCESSION NUMBERS

The NCBI accession numbers for the mRNA sequences for *Salmo salar dio1*, *dio2a*, *dio2b*, *dio3a1*, *dio3a2*, and *dio3b* reported in this paper are KP851703, KP851704, KP851705, KP851706, KP851707, and KP851708, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.01.074.

AUTHOR CONTRIBUTIONS

M.L. performed phylogenetic analysis and gene expression analysis, generated luciferase reporter constructs, and interpreted results. E.C. performed gene expression analysis by qPCR and interpreted results. E.K. performed the microarray experiment and interpreted results. A.D. performed statistical analysis of microarray data. M.J.B. performed in situ hybridization analysis. L.O.E.E. performed and analyzed the iopanoic acid experiment, performed histochemical analysis, and interpreted results. T.O.N. performed and analyzed the iopanoic acid experiment. W.C.J. conceived and designed the study. E.H.J. performed and analyzed the SW exposure experiment. H.D. performed and analyzed the luciferase reporter experiments and interpreted results. D.G.H. and S.A.M.M. conceived and designed the study, analyzed and interpreted results, wrote the manuscript, and gave final approval of the manuscript. All authors discussed and commented on the manuscript.

ACKNOWLEDGMENTS

This study was supported by a grant from the Biotechnology and Biological Sciences Research Council (BB/H008063/1) UK to D.G.H. and S.A.M.M. and the Research Council Norway (project #222215) to L.O.E.E. M.L. was funded by a Marine Alliance for Science and Technology for Scotland PhD studentship. The authors wish to thank Valentina Tronci for excellent technical assistance.

Received: January 28, 2014
Revised: January 9, 2015
Accepted: January 30, 2015
Published: March 19, 2015

REFERENCES

1. Dickhoff, W.W., Folmar, L.C., and Gorbman, A. (1978). Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. *36*, 229–232.
2. Hoar, W.S. (1988). The physiology of smolting salmonids. In Fish Physiology, Vol. XIB, W.S. Hoar and D. Randall, eds. (New York: Academic Press), pp. 275–343.
3. McCormick, S.D. (2001). Endocrine control of osmoregulation in teleost fish. Am. Zool. *41*, 781–794.
4. Stefansson, S.O., Haugland, M., Björnsson, B.Th., McCormick, S.D., Holm, M., Ebbesson, L.O.E., Holst, J.C., and Nilsen, T.O. (2012). Growth, osmoregulation and endocrine changes in wild Atlantic salmon smolts and post-smolts during marine migration. Aquaculture *362*, 127–136.
5. Larsen, D.A., Swanson, P., and Dickhoff, W.W. (2011). The pituitary-thyroid axis during the parr-smolt transformation of Coho salmon, *Oncorhynchus kisutch*: quantification of TSH β mRNA, TSH, and thyroid hormones. Gen. Comp. Endocrinol. *171*, 367–372.
6. Alexandrou, M.A., Swartz, B.A., Matzke, N.J., and Oakley, T.H. (2013). Genome duplication and multiple evolutionary origins of complex migratory behavior in Salmonidae. Mol. Phylogenet. Evol. *69*, 514–523.
7. Macqueen, D.J., and Johnston, I.A. (2014). A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proc. Biol. Sci. *281*, 20132881.
8. Rondeau, E.B., Minkley, D.R., Leong, J.S., Messmer, A.M., Jantzen, J.R., von Schalburg, K.R., Lemon, C., Bird, N.H., and Koop, B.F. (2014). The genome and linkage map of the northern pike (*Esox lucius*): conserved synteny revealed between the salmonid sister group and the Neoteleostei. PLoS ONE *9*, e102089.
9. Darras, V.M., and Van Herck, S.L.J. (2012). Iodothyronine deiodinase structure and function: from ascidians to humans. J. Endocrinol. *215*, 189–206.
10. Nakane, Y., and Yoshimura, T. (2014). Universality and diversity in the signal transduction pathway that regulates seasonal reproduction in vertebrates. Front Neurosci *8*, 115.
11. Hazlerigg, D.G., and Simonneaux, V. (2014). Seasonal regulation of reproduction in mammals. In Knobil and Neill's Physiology of Reproduction, Fourth Edition, T.M. Plant and A.J. Zeleznik, eds. (New York: Academic Press), pp. 1575–1604.
12. Dardente, H., Hazlerigg, D.G., and Ebling, F.J.P. (2014). Thyroid hormone and seasonal rhythmicity. Front. Endocrinol. (Lausanne) *5*, 19, http://dx.doi.org/10.3389/fendo.2014.00019.

13. McCormick, S.D. (2013). Smolt physiology and endocrinology. In Fish Physiology, Volume 32, Euryhaline Fishes, S.D. McCormick, C.J. Brauner, and A.P. Farrell, eds. (Amsterdam: Academic Press), pp. 199–251.

14. Nilsen, T.O., Ebbesson, L.O., Madsen, S.S., McCormick, S.D., Andersson, E., Björnsson, B.Th., Prunet, P., and Stefansson, S.O. (2007). Differential expression of gill Na^+,K^+-ATPase α- and β-subunits, Na^+,K^+,$2Cl^-$ cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*. J. Exp. Biol. *210*, 2885–2896.

15. Ekström, P., Johnsson, C.M., and Ohlin, L.M. (2001). Ventricular proliferation zones in the brain of an adult teleost fish and their relation to neuromeres and migration (secondary matrix) zones. J. Comp. Neurol. *436*, 92–110.

16. Hall, P.A., Levison, D.A., Woods, A.L., Yu, C.C., Kellock, D.B., Watkins, J.A., Barnes, D.M., Gillett, C.E., Camplejohn, R., Dover, R., et al. (1990). Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J. Pathol. *162*, 285–294.

17. Bennett, L., Yang, M., Enikolopov, G., and Iacovitti, L. (2009). Circumventricular organs: a novel site of neural stem cells in the adult brain. Mol. Cell. Neurosci. *41*, 337–347.

18. Ebbesson, L.O.E., Nilsen, T.O., Helvik, J.V., Tronci, V., and Stefansson, S.O. (2011). Corticotropin-releasing factor neurogenesis during midlife development in salmon: genetic, environmental and thyroid hormone regulation. J. Neuroendocrinol. *23*, 733–741.

19. Lema, S.C., and Nevitt, G.A. (2004). Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. J. Exp. Biol. *207*, 3317–3327.

20. Nakao, N., Ono, H., Yamamura, T., Anraku, T., Takagi, T., Higashi, K., Yasuo, S., Katou, Y., Kageyama, S., Uno, Y., et al. (2008). Thyrotrophin in the pars tuberalis triggers photoperiodic response. Nature *452*, 317–322.

21. Ferraris, J.D., Williams, C.K., Ohtaka, A., and García-Pérez, A. (1999). Functional consensus for mammalian osmotic response elements. Am. J. Physiol. *276*, C667–C673.

22. López-Bojórquez, L., Villalobos, P., García-G, C., Orozco, A., and Valverde-R, C. (2007). Functional identification of an osmotic response element (ORE) in the promoter region of the killifish deiodinase 2 gene (FhDio2). J. Exp. Biol. *210*, 3126–3132.

23. Tacchi, L., Bron, J.E., Taggart, J.B., Secombes, C.J., Bickerdike, R., Adler, M.A., Takle, H., and Martin, S.A.M. (2011). Multiple tissue transcriptomic responses to *Piscirickettsia salmonis* in Atlantic salmon (*Salmo salar*). Physiol. Genomics *43*, 1241–1254.

24. Nakane, Y., Ikegami, K., Iigo, M., Ono, H., Takeda, K., Takahashi, D., Uesaka, M., Kimijima, M., Hashimoto, R., Arai, N., et al. (2013). The saccus vasculosus of fish is a sensor of seasonal changes in day length. Nat. Commun. *4*, 2108–2112.

25. O'Brien, C.S., Bourdo, R., Bradshaw, W.E., Holzapfel, C.M., and Cresko, W.A. (2012). Conservation of the photoperiodic neuroendocrine axis among vertebrates: evidence from the teleost fish, *Gasterosteus aculeatus*. Gen. Comp. Endocrinol. *178*, 19–27.

26. Ebbesson, L.O.E., Ekström, P., Ebbesson, S.O.E., Stefansson, S.O., and Holmqvist, B. (2003). Neural circuits and their structural and chemical reorganization in the light-brain-pituitary axis during parr-smolt transformation in salmon. Aquaculture *222*, 59–70.

27. Ebbesson, L.O.E., Björnsson, B.Th., Ekström, P., and Stefansson, S.O. (2008). Daily endocrine profiles in parr and smolt Atlantic salmon. Comp. Biochem. Physiol. A Mol. Integr. Physiol. *151*, 698–704.

28. Stefansson, S.O., Björnsson, B.Th., Ebbesson, L.O.E., and McCormick, S.D. (2008). Smoltification. In Fish Larval Physiology, R.N. Finn and B.G. Kapoor, eds. (Enfield: Science Publishers), pp. 639–681.

29. Dickhoff, W.W. (1993). Hormones, metamorphosis, and smolting. In The Endocrinology of Growth, Development, and Metabolism in Vertebrates, M.P. Schreibman, C.G. Scanes, and P.K.T. Pang, eds. (New York: Academic Press), pp. 519–540.

30. Hanon, E.A., Lincoln, G.A., Fustin, J.M., Dardente, H., Masson-Pévet, M., Morgan, P.J., and Hazlerigg, D.G. (2008). Ancestral TSH mechanism signals summer in a photoperiodic mammal. Curr. Biol. *18*, 1147–1152.

31. Yasuo, S., and Yoshimura, T. (2009). Comparative analysis of the molecular basis of photoperiodic signal transduction in vertebrates. Integr. Comp. Biol. *49*, 507–518.

32. Mazurais, D., Brierley, I., Anglade, I., Drew, J., Randall, C., Bromage, N., Michel, D., Kah, O., and Williams, L.M. (1999). Central melatonin receptors in the rainbow trout: comparative distribution of ligand binding and gene expression. J. Comp. Neurol. *409*, 313–324.

33. Ravi, V., and Venkatesh, B. (2008). Rapidly evolving fish genomes and teleost diversity. Curr. Opin. Genet. Dev. *18*, 544–550.

34. Hazlerigg, D., and Loudon, A. (2008). New insights into ancient seasonal life timers. Curr. Biol. *18*, R795–R804.

35. Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. Physiol. Rev. *84*, 277–359.

36. McDowall, R.M. (1988). Life history deviation and landlocking. In Diadromy in Fishes Migrations between Freshwater and Marine Environments, R.M. McDowall, ed. (London: Chapman & Hall), pp. 71–185.

37. McDowall, R.M. (1997). The evolution of diadromy in fishes (revisited) and its place in phylogenetic analysis. Rev. Fish Biol. Fish. *7*, 443–462.